

Exhibit A

EXPERIMENTAL METHODS

Preparation of p43, 146-amino acid N-terminal and 166-amino acid C-terminal domain of p43

Human pro-EMAPII is genetically separated into the N- and C-terminal domains by proteolytic cleavage at Asp147. The cDNA encoding the full-length pro-EMAPII was isolated from pM3382 by *Nde*I and *Xho*I digestion and then used as a template to separately amplify the DNA encoding its N- and C-terminal domains by PCR using the primer pairs of R1EF/S1ENB and R1ECF/S1EB (Table I). The PCR products were digested and cloned into pET28a using *Eco*RI and *Sal*I.. The resulting clones were transformed into *Escherichia coli* strain BL21-DE3, and the inserted genes were induced at 0.1 mM IPTG. The cells expressing the recombinant proteins were harvested, resuspended in 20 mM KH₂PO₄, 500 mM NaCl (pH 7.8), and 2 mM 2-mercaptoethanol, and then lysed by ultrasonication. After centrifugation of the lysate at 25,000 3 g, the supernatants were recovered and the recombinant proteins containing a 6-histidine tag were isolated by nickel affinity chromatography according to the instructions of the manufacturer (Invitrogen).

<TABLE I>

Primers Sequences

R1EF : 5'-CCGGAATTCATGGCAAATAATGATGCT

R1ECF : 5'-CCGGAATTCTCTAAGCCAATAGATGTT

S1EB : 5'-CCGGTCGACTTATTGATTCCACTGTT

S1ENB : 5'-CTGGTCGACGTCGGCACTTCCAGC

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Skin Wound Generation and Histological Analysis

For *in vivo* wounding experiments, we used 8-week old male C57BL6 mice. We anesthetized mice with an intra-peritoneal injection of 2.5% avertin (100 μ l/10g), shaved the dorsum, cleaned with 70% alcohol for disinfection. A circle of 0.5cm in diameter was marked on the skin of the mid-dorsal region, and the full-thickness excisional wounds including the skin and panniculus carnosus muscle were created using scissors. The wounds were left uncovered without a dressing. One wound was generated per each mouse. After wounding, we sacrificed the mice at time interval for histological analyses. We also treated the wounds with the different forms of p43, 146-amino acid N-terminal of p43 and 166-amino acid C-terminal domain of p43 in PBS with 20% glycerol at the indicated concentration two times a day at 12h interval till the day 3 after wounding. The wound closure was monitored daily using Image-pro Plus software and calculated as the percentage of the initial wound area. We isolated the wounds from the mice, immediately fixed overnight with 10% formaldehyde, dehydrated, embedded in paraffin. We then sliced the embedded tissues by microtome (Leica), mounted them on the silane-coated slides, dewaxed, rehydrated, stained with hematoxylin and eosin, and observed them by microscopy (Nikon TE300).

Immunofluorescence Staining

We fixed the isolated wounds with 4% paraformaldehyde at 4°C overnight, washed with PBS, incubated in 30% sucrose for 4h, and finally froze at -70 °C in optimal cutting temperature (OCT) compound. The frozen sections (6 μ m) were

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attached to the silane-coated slides, treated with PBS, blocked with PBS containing 0.1% Tween 20 and 1% skim milk, and reacted with the antibodies specific to p43, Ki67 (Santa Cruz) at 37 °C for 2h. We washed the slides with PBS containing 0.1% Tween 20 and incubated at 37°C for 1h with the FITC-conjugated secondary antibody. The nuclei were counterstained with propidium iodide (10µg/ml) for 10min and the sections were examined under the confocal immunofluorescence microscopy (μ -Radiance, BioRad).

RESULTS

After introducing the full-thickness excisional wound on dorsal skin of mouse, we treated the wounds with p43 twice a day for three days. We then monitored whether the supplement of p43 would promote the overall wound healing process. On post-wound day 3, the wound areas treated with p43-F or -N were reduced to about 40% of the initial size of the wound whereas those treated with vehicle or p43-C were still about 70% of the initial wounds (Figure A). We sacrificed the mice at 3 day after wounding and checked the proliferation of dermal fibroblasts by immunofluorescence staining with anti-Ki67 antibody that is the marker of proliferation. The full and N-terminal domain of p43 increased the proliferating dermal skin fibroblasts whereas its C-terminal domain did not (Figure B).

Exhibit A

Figure legends

A: The effect of the different forms of p43 on wound closure was evaluated at time interval (**top**) and the relative size of the determined wound area was shown as line graph taking the initial wound size as 100% (**bottom**). The wounds were generated on the dorsal skin of mice as above and treated with the same molar concentration of p43-F (4 µg/wound), p43-N (2.1 µg/wound) and p43-C (2.6 µg/wound) twice a day at post-wounding day 0, 2 and 4, and wound closure was monitored (**top**), and the relative sizes of the wounds were represented as line graph (**bottom**).

B: The wounds were treated with different forms of p43 twice a day for three days (PW 0, 1 and 2). The post-wound 3 day tissues were isolated and the cross sections of the wound area were subjected to H&E staining. (scale bar=1mm). The re-epithelialization regions in the wounds (marked with boxes) were stained with anti-Ki67 antibody and PI to visualize the proliferating fibroblasts (green) and nuclei (red), respectively. (scale bar=25µm) The Ki-67 positive (green) and total (red) cells were counted in the four different wound areas of 0.044mm², and the percentages of the proliferating cells were averaged and demonstrated by bar graphs.